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L17 ANSWER 17 OF 26 USPATFULL

ACCESSION NUMBER:	2000:73929 USPATFULL			
TITLE:	Method of using cross-linked fibrin material			
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PATENT ASSIGNEE(S):	Baxter International Inc., Deerfield, IL, United States (U.S. corporation)			
	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 6074663		20000613	
	WO 9622115		19960725	
APPLICATION INFO.:	US 1997-860864		19970828	(8)
	WO 1996-EP160		19960116	
			19970828	PCT 371 date
			19970828	PCT 102(e) date
	NUMBER		DATE	
PRIORITY INFORMATION:	DE 1995-19501067		19950116	
DOCUMENT TYPE:	Utility			
FILE SEGMENT:	Granted			
PRIMARY EXAMINER:	Brouillette, D. Gabrielle			
LEGAL REPRESENTATIVE:	Wallenstein & Wagner, Ltd.			
NUMBER OF CLAIMS:	54			
EXEMPLARY CLAIM:	1			
NUMBER OF DRAWINGS:	8 Drawing Figure(s); 7 Drawing Page(s)			
LINE COUNT:	1563			

having a pore size below 1 .mu.m (about 0.2 .mu.m). For comparison, FIG. 3 shows a representative human fibrin clot structure. In the human haemostatic clot, the presence of cells drastically opens the three-dimensional structure of the network. Such an opened and irregular structure is physiologically favorable to **fibroblast migration** into the fibrin clot network during the normal wound healing process. It is apparent from the figures that by varying the thrombin concentration, fibrin networks with low or high pore size are obtainable. For the use of the fibrin material as a bio-mechanical barrier in accordance with the present invention, the thrombin concentration is preferably adjusted to obtain a fibrin network structure with a pore size excluding fibroblast penetration. The fibrin material produced in accordance with the invention may be examined by standard SEM and further be tested in the animal model described herein.

SUMM Under consideration of these three factors, in certain embodiments haemostasis and wound repair is addressed by applying a single layer of fibrin glue to the injury site(s), while the separation/isolation of the injured surface(s) is achieved by using a bio-mechanical fibrin barrier, either applied as a second layer on top of the first layer of the fibrin glue, or simply as a self-supporting sheet placed between the adjacent injury sites or between the site of the injury and the adjacent uninjured tissues. The inventors have discovered that an important parameter to be taken into account in using such a combination of a haemostatic agent/wound repair promoter and a bio-mechanical barrier is the time required for complete conversion of **fibrinogen** to fibrin. Specifically, it has been found that the layer of the fibrin glue and respectively the last layer, if more than one layer is applied to an injured surface, should be allowed to set until the conversion of **fibrinogen** to fibrin is complete. By way of example, when fibrin glue is applied simultaneously to two injured surfaces such as caecum and peritoneal wall in order to form a single layer each time, and the surfaces come into contact with each other before the **fibrinogen** -fibrin conversion is complete, it may occur that these surfaces are glued together, i.e., that adhesions are formed.

SUMM This means that the surface state of the different interfaces and their relationships are very important in the prevention of adhesions. As a general guidance, the inventors propose to allow undisturbed setting after application of the respective last external layer of fibrin glue until the conversion of **fibrinogen** to fibrin is complete. This does not apply to the fibrin film of the invention, since this is allowed to set completely in vitro before application. Of course, although detailed experimental protocols are described hereinafter, those of skill in the art will appreciate that the specific time requirements may vary depending on the particular patient, the type of injury and the handling, and is thus apparently also a matter of clinical experience. However, in vitro methods are known in the art for monitoring the **fibrinogen**-fibrin conversion. By way of example this can be followed by monitoring turbidity which is the measure of the optical density of fibrin networks developed in a cuvette with a path of one centimetre at 800 nm (cf. G.A. Shah et al., Thrombosis Research 40, 818-188, 1985). In accordance with this method it is possible to determine in vitro the time required for complete fibrin formation at a given thrombin concentration. This provides an estimate of the minimum time required for complete setting after application of the last external layer(s). It is believed that, based on the present disclosure, one of average skill in the art could define a protocol for use of a dedicated fibrin glue, its mode and type of application, so that the requirements for surgeons with respect to the timing and the technical devices are met.

SUMM In accordance with the general guidelines described above, a preferred embodiment called "double coating" comprises the application of a first

fibrin glue with a low concentration of thrombin to work as haemostatic agent and/or tissue repair promoter, and of a second fibrin glue with a to high concentration of thrombin playing the role of a bio-mechanical barrier which entirely covers the injury and the first coating formed upon application of the first fibrin glue. Preferably the first fibrin glue has been made by mixing of the above-described **fibrinogen** -containing solution with an equal volume of a thrombin-containing solution comprising less than 1000 IU thrombin, preferably less than 150 IU. The fibrin glue has been preferably made by mixing said **fibrinogen**-containing solution with an equal volume of a thrombin-containing solution of at least 50 IU thrombin, preferably of at least 150 IU thrombin, and most preferably of least 300 IU thrombin. Of course, it will also be possible to apply more than two layers as long as the last layer plays the role as a bio-mechanical barrier preventing fibroblast proliferation between the covered lesion and the adjacent surfaces.

SUMM In another preferred embodiment of the invention called "sandwich method", a fibrin glue layer covering the injured surface(s) is used as haemostatic agent and wound repair promoter, while a fibrin film, in i.e., a self-supporting sheet-like material of cross-linked fibrin being placed between the injured surface and an adjacent uninjured surface, or between two injured surfaces, acts as a bio-mechanical barrier. The fibrin glue is preferably produced by mixing of a first, **fibrinogen**-containing solution with an equal volume of a thrombin-containing solution comprising 1-300 IU/ml thrombin, preferably at least 20 IU/ml thrombin and most preferably at least 100 IU/mi thrombin. The fibrin film is made of at least 4 IU/ml thrombin, preferably of at least 20 IU/ml thrombin, and most preferably of at least 300 IU/ml thrombin. It will, of course, be recognized that the fibrin film can also be used in combination with a double coating as described above.

DETD

Vial (1)	Human topical **fibrinogen** complex (dry concentrate)	
	protein	10-13 g/100 ml
	clottable protein	80% minimum
	albumin (human)	0,5-1,5 g/100 ml
	plasminogen	0,05 mg/ml maximum
	Factor XIII	10-40 IU/ml
	polysorbate-80	0,3 % (w/v) maximum
	pH	7,1-7,5
Vial (2)	Sterile water (3,5 ml) for reconstituting the content of vial (1) at 37.degree. C. in a water bath	
Vial (3)	Human thrombin	
	potency	300 .+-. 50 IU/ml
	albumin (human)	0,05 .+-. 0,01 g/ml
	glycine	0,30 M .+-. 0,05 M
	pH	6,5-7,1
Vial (4)	35-45 mM CaCl.sub.2 (3,5 ml) for reconstituting the content of vial (3) at room temperature	

DETD After reconstitution, the **fibrinogen**-containing solution was kept at room temperature. Further thrombin dilutions were made with 20 mM CaCl.sub.2 as diluent. Using the dual syringe device, the mixture " **Fibrinogen**-Thrombin" was applied to a petri dish, while care was taken that at any time equal amounts of the **fibrinogen** -containing solution and the thrombin-containing solution were pressed

out of the respective syringe. With low concentrations of thrombin, the petri dish was tilted to cover the surface with a fibrin glue of regular and homogenous thickness. With high concentrations of thrombin, particular care was taken that from the beginning the mixture of the **fibrinogen**-containing solution and the thrombin-containing solution was uniformly spread over the surface of the petri dish. The petri dish was incubated at 37.degree. C. for two hours.

DETD Preparation of a Fibrin Film Using a Dry **Fibrinogen** Sheet

DETD 3.5 ml of the reconstituted **fibrinogen**-containing solution were poured in a petri dish of 51 mm diameter which was tilted to spread the material all over the entire surface. The water contained in the **fibrinogen**-containing solution was evaporated by air drying. Thus, a dry **fibrinogen** sheet having a thickness of 100 .mu.m and a weight of 0.4291 g was obtained. 3.5 ml of a reconstituted thrombin-containing solution were poured into the petri dish containing the dry, sheet-like **fibrinogen** material. The reaction mixture was then kept at 37.degree. C. for 2 hours. The fibrin film thus obtained may either directly be used or be dried and rehydrated before use. Alternatively, the dry, sheet-like **fibrinogen** material may be converted into a fibrin film only before use.

DETD Both the dry, sheet-like **fibrinogen** material and the dried fibrin film may be included in a commercial kit further comprising ancillary components for processing and rehydration, respectively, of the sheet-like materials.

DETD The first, **fibrinogen**-containing solution was poured in a petri dish having a diameter of 91 mm. The temperature of said solution was decreased bating the petri dish for a few minutes at low temperature, here for 4 min at -12.degree. C. Then the second, thrombin-containing solution (RT) was added and mixed with the first solution. The petri dish was incubated until completion of the conversion of **fibrinogen** to fibrin, here for 24 hours at 37.degree. C.

DETD The sandwich method combines the use of a fibrin glue as haemostatic agent/wound repair promoter and of a fibrin film as mechanical barrier. Three types of fibrin film which had been made using 4 IU, 20 IU and 300 IU thrombin in accordance with Example 1 were used. Due to the different thrombin concentrations of the respective fibrin films, the time required for complete **fibrinogen**-fibrin conversion varied. However, this is of no importance as the films were kept at 37.degree. C. for more than two hours, a time greater than that required as determined theoretically and practically by means of turbidity measurement.

DETD Sequential application of FG 4 IU (cf. Example 6a) did not prevent adhesion formation at all, whereas sequential application of FG 100 IU (cf. Example 6b) allowed a 50% prevention of adhesion formation. These findings suggest that the conversion of **fibrinogen** to fibrin was not complete on both caecum and parietal wall.

DETD In Example 7b, rather than to increase the simultaneous application time/waiting time for achieving complete **fibrinogen**-fibrin conversion, the thrombin concentrations were increased in order to reduce the clotting time. In fact, with the simultaneous application of FG 5 & 150 for five minutes, the number of adhesions was decreased.

DETD The presence of a remaining fibrin piece in Examples 7a and 7b indicates, however, that the application volume had to be better controlled. It also has to be pointed out that the double coating of fibrin glues was applied to an injured area where the fibrinolytic system was dramatically impaired. A more controlled delivery (by better handling) and a lower volume of fibrin glue at a higher thrombin concentration (to achieve a faster and more complete conversion of **fibrinogen** to fibrin) seem to be more suitable for improving the outcome.

DETD As shown in Table 8, simultaneous application of FG 100 IU/FF 4 IU/FG 100 IU for five minutes did not totally prevent adhesion formation. The fibrin film made by using 4 IU thrombin is indeed a stabilized film

having a complete **fibrinogen**-fibrin conversion, but the inventors are aware that such a fibrin film has particularly large and opened pores. On the other hand, Table 8 shows that FG 100 IU, applied with a waiting time of five minutes, did not completely prevent the development of adhesions. Thus, it may be that FG 100 IU had not reached completion of the conversion of **fibrinogen** to fibrin and interacted with FF 4 IU in such a manner that no complete prevention of adhesion formation was achieved.

DETD Principally, this could be avoided by either increasing the thrombin concentration of the fibrin glue used (to achieve a faster clotting) or by increasing its sequential application time (to provide more time for the **fibrinogen**-fibrin conversion), or by increasing the thrombin concentration of the fibrin film (to produce smaller pores).

CLM What is claimed is:

1. A method of using a self-supporting film material of cross-linked fibrin for the preparation of a medicament for the prevention of adhesion formation as a post-operative complication, comprising the step of applying the film material of cross-linked fibrin to an injured surface, wherein the film material of cross-linked fibrin is prepared by the steps comprising: (a) converting **fibrinogen** to fibrin using a thrombin-containing solution having a concentration of at least 20 IU/ml; (b) converting the **fibrinogen** such that the conversion to fibrin is substantially complete in that there is essentially no unreacted **fibrinogen** in the fibrin material; and (c) forming a fibrin material having a pore size of below 5 .mu.m.

6. The method of claim 5, wherein the fibrin glue applied to the injured surface has been made by mixing a **fibrinogen**-containing solution having a factor XIII content of 10-40 IU/ml with an equal volume of a thrombin-containing solution comprising 1-300 IU/ml thrombin, and calcium; said **fibrinogen**-containing solution being a protein solution with a content of 90-140 mg protein/ml comprising up to 90% clottable protein.

7. The method of claim 5, wherein the fibrin glue is allowed to set undisturbed on the surface applied to until the conversion of **fibrinogen** to fibrin is complete.

11. A process of preparing a self-supporting film material of cross-linked fibrin, said process comprising the steps of: a. simultaneously mixing a stream of a first, **fibrinogen** -containing solution with a stream of a second, thrombin-containing solution having a concentration of at least 20 IU/ml; b. applying the obtained mixture to a solid support; c. incubating the mixture until the conversion of **fibrinogen** to fibrin is substantially complete such that there is essentially no unreacted **fibrinogen** in the fibrin material; and d. forming a fibrin material having a pore size of below 5 .mu.m.

14. The process of claim 11, wherein the first, **fibrinogen** -containing solution has a factor XIII content of 10-40 IU/ml, and the second, thrombin-containing solution has a thrombin content of 20-300 IU/ml, and a calcium content of up to 45 mM, said first **fibrinogen**-containing solution being a protein solution with a content of 90-140 mg protein/ml comprising up to 90% clottable protein.

18. A process of preparing a self-supporting film material of cross-linked fibrin, said process comprising the steps of: a. applying a first, aqueous, **fibrinogen**-containing solution having a factor XIII content of 10-40 IU/ml and a protein content of 90-140 mg protein/ml comprising up to 90% clottable protein to a solid support; b. removing water until dryness, forming a sheet-like **fibrinogen** material; c. applying to the sheet-like **fibrinogen** material a second, thrombin-containing solution having a thrombin content of 20-300

IU/ml and a calcium content of up to 45 ml; and d. incubating until the conversion of **fibrinogen** to fibrin is substantially complete such that there is essentially no unreacted **fibrinogen** in the fibrin material to form said fibrin material having a pore size of below 5 .mu.m.

22. The process of using a first fibrin glue acting as a haemostatic agent in combination with a second fibrin glue acting as a bio-mechanical barrier for the preparation of a medicament for the prevention of adhesion formation as a post-operative complication, wherein the second fibrin glue is prepared by the steps comprising: (a) converting **fibrinogen** to fibrin using a thrombin-containing solution having a concentration of at least 20 IU/ml; (b) converting the **fibrinogen** such that the conversion to fibrin is substantially complete in that there is essentially no unreacted **fibrinogen** in the fibrin material; and (c) forming a fibrin material having a pore size of below 5 .mu.m.

25. The process of claim 22 wherein the first fibrin glue has been made by mixing a **fibrinogen**-containing solution having a factor XIII content of 10-40 IU/ml and a protein content of 90-140 mg protein/ml comprising up to 90% clottable protein with an equal volume of a thrombin-containing solution comprising less than 1000 IU/ml thrombin and the second fibrin glue has been made by mixing said **fibrinogen**-containing solution with an equal volume of a thrombin-containing solution comprising at least 50 IU/ml thrombin and calcium.

52. The process of claim 25 wherein the **fibrinogen**-containing solution is autologous.

54. The process of claim 25 wherein the **fibrinogen**-containing solution and the thrombin-containing solution are autologous.

L17 ANSWER 18 OF 26 USPATFULL

L10 ANSWER 1 OF 2 USPATFULL
ACCESSION NUMBER: 97:78178 USPATFULL
TITLE: Anti-scarring compositions comprising growth factor neutralizing antibodies
INVENTOR(S): Ferguson, Mark William James, Stockport, England
Foreman, David Michael, Chorlton, United Kingdom
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PATENT ASSIGNEE(S): The Victoria University of Manchester, Manchester, England (non-U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 5662904	19970902	
	WO 9217206	19921015	
APPLICATION INFO.:	US 1993-122508	19930927	(8)
	WO 1992-GB570	19920330	
		19930927	PCT 371 date
		19930927	PCT 102(e) date

	NUMBER	DATE
PRIORITY INFORMATION:	GB 1991-6678	19910328

DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Scheiner, Toni R.
ASSISTANT EXAMINER: Johnson, Nancy A.
LEGAL REPRESENTATIVE: Wallenstein & Wagner, Ltd.
NUMBER OF CLAIMS: 28
EXEMPLARY CLAIM: 1,2
LINE COUNT: 788
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L10 ANSWER 2 OF 2 USPATFULL
ACCESSION NUMBER: 90:23593 USPATFULL
TITLE: Use of synthetic sulfated saccharides to enhance wound healing
INVENTOR(S): Michaeli, Dov, San Francisco, CA, United States
PATENT ASSIGNEE(S): Marion Laboratories, Inc., Kansas City, MO, United States (U.S. corporation)

	NUMBER	DATE	
PATENT INFORMATION:	US 4912093	19900327	
APPLICATION INFO.:	US 1986-922358	19861023	(6)

RELATED APPLN. INFO.: Continuation-in-part of Ser. No. US 1986-914192, filed on 1 Oct 1986, now abandoned which is a continuation-in-part of Ser. No. US 1985-813243, filed on 24 Dec 1985, now abandoned
DOCUMENT TYPE: Utility
PRIMARY EXAMINER: Brown, Johnnie R.
ASSISTANT EXAMINER: Peselev, Elli
LEGAL REPRESENTATIVE: Gillis, Theresa M.
NUMBER OF CLAIMS: 21
EXEMPLARY CLAIM: 1
LINE COUNT: 973
CAS INDEXING IS AVAILABLE FOR THIS PATENT.